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Dear Ulf,

How can I start my letter! I feel so guilty about not having written in so long that I can only place myself at your mercy and hope you'll forgive me. The most charitable thing you could do is to accept that I'm a very poor correspondent.

When your letter of December 1962 arrived I was so immersed in my upcoming teaching obligation that I put off answering until I had my lectures finished. As you may know, it was my turn to give the graduate course (once every 3 years) and for reasons which I will never forgive myself for, I chose to talk about protein structure as related to its function. In particular, I decided to cover in detail pancreatic RNase, its sequence determination, secondary and tertiary structure, modifications of each and the effects on enzyme activity etc. etc. Since I knew very little of the literature and moreover was not up to the minute in protein physical chemistry, I had much to learn. The course ran 10 weeks and I had to give a 2-1/2 hour lecture each week. I spent almost all my time reading, studying and preparing and did little if anything else. I also spent 2 weeks discussing "feedback" control through alteration in protein structure, another area in which I had lots of preparation. At the end of this course, although I knew I had learned an enormous amount, I wondered whether it was worth it.

During this whole period I kept putting off all the other things I was supposed to be doing and planning to catch up when I finished teaching. By March 15 I was exhausted and the trip to Hawaii seemed very appealing as a way to rest up. I was only supposed to give several lectures and talk to people during the six-week stay. It did turn out to be very pleasant and relaxing for Milly, Johnny and me. In fact it was so relaxing that I did very little in the way of catching up on all I had left sit including the long letter I had intended to write to you! We had a lovely house on the beach and while Johnny went to school every day Millie could relax. I did try surfing without much success, although

I enjoyed the effort. When I came back I had five hectic days to prepare for an N.I.H. study section meeting in the East and I was gone a week. On returning Mike and Bill had to get manuscripts ready for the Cold Spring Harbor Symposium talks and that proved a nuisance and was quite time-consuming. The Cold Spring Harbor meeting was pretty hectic but interesting (I'll mention some of the things that might interest you in a moment). Now that I'm back in the lab again and things have eased off I'm trying to get our research started. So you see since last January I've been very busy, although I don't deny that had I been better organized things would not have been so hectic. In fact, I did take the address at which you could be reached to Hawaii.

I think Karl Muench told me that you've had your new baby. I can't remember if he told me whether it was a boy or girl and your last letter didn't say. Your remark about Anna nagging you about coming back to California was of interest - when will you came again? You know that you'll both always be welcome. Maybe you'll be able to come to the International Congress in New York next summer. I'm not sure but you might be on the invitation list. If so, we can have a reunion since Milly is planning to go with me then.

How is Peter? He must be about 3 years old now and full of mischief. Johnny has matured tremendously (or so it seems to me) and will be going to school in September. Milly, who has her hands full trying to keep up with him, is looking forward to the few hours of peace and quiet while he's at school.

As I mentioned earlier, I just got back from Cold Spring Harbor so I can at least relay some new information you'll be interested in. However, I saw Peter Reichard there and he seemed to be taking good notes so he can fill you in on any details I omit.

With respect to S-RNA, Holley and Ingram are continuing their studies of the fragments produced by RNases on purified (homogeneous?) S-RNA's and while they have made considerable progress nothing of clear interest has yet emerged except that some S-RNA fractions have considerably higher levels of methylated bases than the average. Cantoni presented a full base sequence of serine RNA but it was pure fantasy and doesn't deserve any more comment.than that. Spencer and Wilkins reported on the X-ray diffraction work of S-RNA and spread a cloud of suspicion over their original conclusion of complete helical structure. It seems that they are looking at degraded RNA not native S-RNA in their "crystalline fibers".

Jerry Hurwitz reported on the RNA and DNA methylases. The methylases (4 separate enzymes for RNA and 1 for DNA) each methylate a specific base in RNA or DNA and interestingly enough Jerry reports a striking species specificity between substrate and enzymes. For example, enzymes from one bacterial species will not methylate its own S-RNA but will methylate that of another. Unmethylated RNA (prepared in Borek's mutant strains on meth) will accept methyl groups from its homologous enzyme. The significance of the methyl groups with

respect to biologic activity is still in doubt, however, since Uri Littauer, Karl Muench and I did some experiments to show that their absence does not affect the ability to accept nor the specificity of acceptance. Moreover, amino acyl (-demethylated) S-RNA seems to transfer amino acid to the ribosomes as efficiently as does the fully methylated counterpart.

Von Ehrenstein and Benzer have reported some experiments that were interesting. Whereas the different leucine-specific S-RNA will transfer leucine to different copolymers (published data) they transfer leucine to the identical sites of haemoglobin or MS 2 phage protein. Similarly two serine transfer RNA peaks transfer serine to some Ser Arg peptide of f2 phage protein. One wonders now how valid are the "decoding" experiments of Nirenberg and Ochoa. Also Yamane and Sueoka reported on studies very much like those described in your paper.

I shall (Carolyn says they've gone off) send you the manuscripts of Mike's and Bill's papers so that you can see what we reported. I've done very little more on the sequence studies using mixed polymers although Harriet and I are getting back to that now.

Let me get to my comments about your manuscript. I didn't get a chance to read it before I left for CSH but since returning I've read it very carefully. I have many questions, probably some of which you recognized, but I'll write them down in detail.

1) p. 3. I was puzzled by the standard assay conditions you describe. First, the amount of amino acid you use ($2 - 4 \times 10^{-5}$M) seems to be below the saturating value for valine and Ø-alanine quoted on page 10. Moreover, on page 10 where you say that ratios of Mg/ATP >20 were inhibitory you say that the saturating level for ATP is 10^{-4}M and for Mg^{++} is 10^{-2}M; this is a ratio of 100, so the two statements don't correspond! Also, for the optimal values you give on pages 10-11, it's never clear whether these are optimal for rate or yield measurements, or both.

2) p. 11 - 13. I think the data on rates and yield comparisons (Tables 3 and 4) are very nice and show the specificity very clearly.

3) What seems to me the weakest part of the paper (and potentially the most interesting) is the chromatographic work. The results are pretty confusing and one doesn't really know why. This all had to do with the reproducibility of the chromatograms which you discuss but unfortunately doesn't help.

(a) Fig. 1 looks good and is clean. Seems to show that both enzymes load some chains. Legend mentions yeast RNA but does not appear in Figure.

(b) Fig. 2. Here the pattern of label suggests same as above except why is OD pattern so much different here from almost all other?

(c) Fig. 3. Looks OK and discrepancy in region of small second peak is clear.

(d) Fig. 4. Interesting as it stands and I can accept the aggument that discrepancy is not due to contamination of Ø-alanine by other labeled AA. However, you may note in legend that the ^{3}H Ø-alanine RNA coli made by coli enzyme does not have specific activity shown in Table 3 (0.11 instead of 0.30) whereas the other C^{14} Ø-alanine RNA coli made with yeast does.

(e) Fig. 5. Why doesn't valyl RNA yeast look like the one in Fig. 2? Actually if you compare valyl RNA yeast made with yeast in Fig. 2 with valyl RNA made with coli in Fig. 1, there is not too much difference except for displacement in their positions on the column.

(f) Fig. 6. Again has that funny OD profile.

(g) Fig. 7. Why doesn't the Ø-alanine RNA coli made with coli look like the curve shown in Fig. 4? Actually it's exactly the same material.

Because of the apparent difficulty in reproducing the results, I would worry about too strong a conclusion as to whether the same chains are being loaded by the two enzymes. I realize that by co-chromatography the two RNA's one is trying to minimize the difficulties of reproducing the patterns, but I would' still be worried. If I'm not mistaken, Yanane and Sueoka have tried to do the same experiment (but using crude extracts as enzyme source) and they have also run into difficulties in interpretation. Without having had too much experience with the Hershey columns I didn't make any good suggestions for improving, but I know that Dave Hogness has often remarked about how careful one has to be with all conditions to get reproducible results. Have you ever determined whether after loading amino acid with the different enzymes whether you can remove the AA and still load the original amount?

In the main I think the paper is good and I can tell how hard you've worked on these experiments. Have you heard from the JMB about them?

Well, I hope this will make up in part for the long lapse in writing and things should be well enough organized for awhile so that I can hope that it won't happen to this extent again! Hope this finds all of you well and having a nice summer after all the cold weather.

Sincerely,

Paul Berg

PB:cm
Enclosure